Substrate Effects on O_2 Activation by Benzoate Dioxygenase

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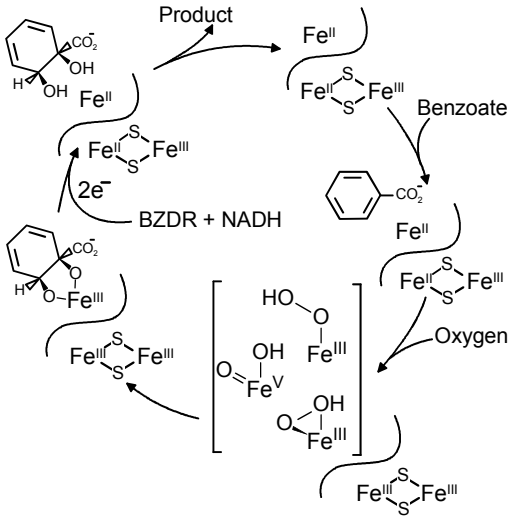
Benzoate dioxygenase (BZDOS, is a multicomponent Rieske non-heme dioxygenase that catalyzes the NADH dependent *cis*-dihydroxylation of benzoate. This enzyme was purified from the native organism *P. putida* and characterized by kinetic and spectroscopic methods.[1] Results from those studies indicate that the reduced oxygenase component (BZDO) alone is sufficient for O_2 activation and substrate dihydroxylation in a single turnover as illustrated in the catalytic cycle shown below. During the single turnover, both the Rieske cluster and the mononuclear iron in the site where O_2 is activated become oxidized, thereby providing the two electrons required by the reaction stoichiometry. It was also reported that O_2 activation is substrate dependent in two ways:

- The substrate must be bound before the enzyme activates dioxygen.
- The rate of electron transfer from the Rieske center to the mononuclear iron depends upon the substrate structure.



The basis for these substrate effects and their bearing on the mechanism of O_2 activation are being addressed by combining transient kinetic studies of electron transfer with mutagenesis of key active site residues that alter the position or dynamics of substrate binding and oxidation. The ability of BZDOS to accept a wide range of substrates allows a systematic evaluation of the effects of substrate size and substituents on electron transfer. A model for the coupling of substrate binding to electron transfer as the basis for the ability of BZDOS to activate O_2 without uncoupling will be presented.

(1)Wolfe, M. D.; Altier, D. J.; Stubna, A.; Popescu, C. V.; Münck, E.; Lipscomb, J. D. *Biochemistry* **2002**, *41*, 9611-9626